

Identification and characterization of bacterial isolates associated with the abdomen of *Anopheles gambiae* s.l. Linneus (Diptera: Culicidae) mosquitoes

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Abstract

Mosquitoes are recognized for their role in the transmission of several parasitic and microbial diseases. This research was conducted to identify and characterize the diversity of microbes inhabiting the abdomen of *Anopheles gambiae* s.l. Linneus (Diptera: Culicidae) due to their possible impact on the vector's ability to transmit *Plasmodium* species to man. Adult *Anopheles gambiae* s.l. Linneus (Diptera: Culicidae) were collected from houses in Rumuepirikom Town, Rivers State, Nigeria, identified using keys and examined for bacterial species using standard microbiological procedures. Nine bacterial species were identified, including *Pseudomonas* sp., *Staphylococcus* sp., *Streptococcus* sp., *Acinetobacter* sp., *Klebsiella* sp., *Bacillus* sp., *Serratia* sp., *Escherichia* sp., and *Enterobacter* sp. Of these, *Pseudomonas* sp., *Serratia marcescens*, *Staphylococcus* sp., *Acinetobacter* sp., *Klebsiella* sp., *Bacillus* sp., and *Enterobacter cloacae* were isolated from the blood-fed mosquitoes while the non-blood fed group harboured *Staphylococcus* sp., *Streptococcus* sp., *Acinetobacter* sp., *Klebsiella* sp., *Bacillus* sp., and *Escherichia coli*. Total Heterotrophic Bacteria Count (THBC) of *Anopheles gambiae* s.l. Linneus (Diptera: Culicidae) sampled ranged from 6.34±0.23 to 6.68±0.10 (LogCFU/ml) and *Salmonella* sp count was zero count. The bacterial count for blood fed 1, blood fed 2, blood fed 3 and non-blood fed *Anopheles gambiae* s.l. Linneus (Diptera: Culicidae) mosquito recorded are 6.34±0.23, 6.63±0.24, 6.68±0.10 and 6.46±0.17 (LogCFU/ml) with *p*-value > 0.05. Two one-sided tests (TOST) analysis revealed inequivalence in the bacterial load between blood-fed and non-blood fed. This research identified the bacterial species associated with the abdomen of *Anopheles gambiae* s.l. Linneus (Diptera: Culicidae) mosquito populations, which are mostly pathogenic gram-negative rods, and there is disparity in the microbial load between blood fed and non-blood fed *Anopheles gambiae* s.l. Linneus (Diptera: Culicidae) mosquitoes.

Keywords: Abdomen, *Anopheles gambiae* s.l., Total Heterotrophic Bacteria Count, Blood fed, Gram-negative bacteria

Introduction

Mosquitoes are vectors of diseases of public health importance including malaria, yellow fever, dengue fever, encephalitis and filariasis, of these, malaria is by far the most important (Service, 1993). *Anopheles* mosquitoes are particularly prominent for their role in the transmission of malaria (Cox, 2010).

However, some factors impact on the ability of mosquito vectors to transmit parasites by limiting the ability of Anopheline mosquito vectors to support development of the malarial parasite in the midgut and through to the infective sporozoite stage in their salivary glands (Sharma *et al.*, 2014). For instance, commensal bacteria in the abdomen can suppress parasite development and reduce the ability of mosquitoes to transmit the parasite to a new host, either by having direct anti-plasmodial effects or by stimulating basal immune responses of the mosquito against parasite development (Osei- Pokuj *et al.*, 2012; Dong *et al.*, 2019). Also, midgut microbiota can affect the development of *Plasmodium* parasites (Cirimotich *et al.*, 2011; Bahia *et al.*, 2014). Bando *et al.*, 2013, reported that isolates of *Enterobacter amnigenus* and *Enterobacter cloacae* are able to impair the sporogenic development of *P. vivax*, while *Serratia marcescens* completely inhibited *P. vivax* development in *Anopheles albimanus*.

Studies have reported on the presence of several microbial species (including *Pseudomonas cepacia*, *Enterobacter agglomerans*, *Escherichia coli* and *Flavibacterium* spp.) in the midgut of both laboratory-reared and field Anopheline

mosquitoes (Straif *et al.*, 1998). Most reports indicate that the most frequent bacterial species in mosquito abdomens are gram negative rods, such as *Serratia marcescens*, *Klebsiella ozaenae*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterobacter* sp. (Manguin *et al.*, 2013).

The interaction between insects and microbes has been studied in a variety of vector species; however, little is known about the bacteria communities associated with the abdomen of mosquitoes (Sharma *et al.*, 2014). This study was hence designed to isolate, identify and characterize the micro-organisms found in the abdomen of *Anopheles gambiae* s.l. Linneus (Diptera: Culicidae) mosquitoes obtained from a location in Rivers State - Nigeria.

Materials and Methods

Study Area

Adult mosquitoes were obtained from three families in Rumuepirikom Town in Obio-Akpor Local Government Area, Rivers State, Nigeria. It is located on the intersection of Latitude 4° 49'45. 6" N Longitude 6° 58' 51.0" E with a temperature of 35°C and relative humidity of 80%. Its geology comprises basically of alluvial sedimentary basin and basement complex. The Rumuepirikom Town is located in the heart of Port Harcourt city and densely populated.

Sample Collection

Four houses were selected for the night sampling of adult *Anopheles* mosquitoes using an aspirator. Mosquitoes were aspirated as soon as they landed on the exposed body parts

such as the feet and knees of each volunteer using a flashlight and mouth aspirator (Berhanu *et al.*, 2019). The following morning, mosquitoes were transported to the insectary of Malaria Vector Surveillance and Insecticide Resistance Monitoring Laboratory of the Department of Animal and Environmental Biology, Rivers State University, Port Harcourt, Nigeria.

Morphological Identification of *Anopheles gambiae* s.l.

Members of *Anopheles gambiae* s.l. were morphologically separated from other anopheline mosquitoes using the morphological identification keys of Gillies and De-Mellion (1968) and Gillies and Coetzee (1987).

Dissection of Mosquitoes

A total of 40 mosquitoes were pooled together in paper cups covered with net as a lid, from the four houses and were anesthetized using sterile cotton wool soaked with acetone. Dissection was accomplished under sterile conditions. The dissection procedure was completely carried out in the laminar flow, to avoid microbial contaminations from the environment. The dissecting stereomicroscope (2x) was cleaned, while the working area, the dissecting scalpels and forceps were dipped in 70% ethanol every time they were to be used in the dissection process (Berhanu *et al.*, 2019).

Microbiological Analysis of *Anopheles gambiae* s.l. Mosquito Samples (Isolation and Enumeration of Bacteria)

The abdominal samples of *Anopheles gambiae* s.l. mosquito sample were aseptically dissected and introduced into a test tube containing 9 ml of sterile H₂O and shaken vigorously for a homogenous suspension to form a stock solution. 1 ml of the stock was thereafter aseptically transferred into 9 ml of sterile H₂O to give 10⁻¹ dilution. Bacteria count of the *Anopheles gambiae* s.l. mosquito samples were determined using spread plate technique. 0.1ml aliquot was plated in duplicates on sterile Nutrient Agar (NA) (28g of the nutrient agar powder weighed into 1000 ml of distilled water) and Salmonella Shigella Agar (SSA) (63g of the Agar weighed into 1000 ml of distilled water) sterilized by autoclaving at 15psi pressure, at 121°C for 15 minutes, according to manufacturer's instructions. The plates were incubated at 37°C for 24 - 48 hours. The bacterial colonies (30-300) that develop after the incubation period were counted and recorded and expressed as Colony Forming Units per millimeter (CFU/ml) of the mosquito sample. The streak plate technique was used to obtain a pure culture by sub-culturing and isolates were preserved and maintained on nutrient agar slants in Bijou bottles stored in the refrigerator at 4°C for further use (Ngo *et al.*, 2016; Oliveira *et al.*, 2020; Tainchum *et al.*, 2020).

Identification and Characterization of Bacterial isolates

The characterization and identification of the bacterial isolates was done based on colonial morphology, cellular morphology and biochemical test carried out to verify the identity of the organisms. The bacterial isolates were identified, and confirmatory identities of bacteria were made using Bergey's manual of determinative bacteriology (Tambuwal *et al.*, 2018; Poly *et al.*, 2022; Ulfat *et al.*, 2022).

Results

The mosquitoes collected from the study location were identified as *Anopheles gambiae* s.l. The results of the

bacterial count (specifically THBC and Salmonella count) and identification of bacterial isolates from the abdomen of *Anopheles gambiae* s.l. were reported.

Enumeration of bacterial counts of *Anopheles gambiae* s.l mosquitoes' abdomen

Total Heterotrophic Bacteria Count (THBC) of *Anopheles gambiae* mosquitoes sampled ranged from 6.34±0.23 to 6.68±0.10 (LogCFU/ml) and Salmonella count was zero count. The bacterial count for blood fed 1, blood fed 2, blood fed 3 and non-blood fed *Anopheles gambiae* mosquito are as follows: 6.34±0.23, 6.63±0.24, 6.68±0.10 and 6.46±0.17 (LogCFU/ml), respectively (Table 1). The blood fed *Anopheles gambiae* mosquitoes recorded the highest THBC than the non-blood fed *Anopheles gambiae* mosquitoes. Statistically, there is no significant difference in the means THBC of the samples (blood fed and non-blood fed) as *P*-value > 0.05.

Identification of bacteria isolates from the abdomen of *Anopheles gambiae* s.l mosquitoes

A total of nine (9) bacterial genera were identified in the abdomen of the *Anopheles gambiae* s.l. mosquitoes, namely *Pseudomonas* sp. 2(16.67%), *Staphylococcus* sp. 2(16.67%), *Streptococcus* sp. 1(8.33%), *Acinetobacter* sp. 1(8.33%), *Klebsiella* sp. 1(8.33%), *Bacillus* sp. 1(8.33%), *Serratia* sp. 2(16.67%), *Escherichia* sp. 1(8.33%), and *Enterobacter* sp. 1(8.33%). The species isolated from the blood-fed mosquitoes were *Pseudomonas* sp, *Serratia marcescens*, *Acinetobacter* sp., *Klebsiella* sp., *Bacillus* sp., and *Enterobacter cloacae*. The non-blood fed group harboured *Streptococcus* sp., *Acinetobacter* sp., *Klebsiella* sp., *Bacillus* sp., and *Escherichia coli*. *Staphylococcus* sp. was isolated from both blood fed and non-blood fed *Anopheles gambiae* s.l. mosquitoes

The Gram's reaction indicated that out of the twelve (12) characterized bacteria, nine (9, 75%) isolates were Gram negative rods, two (2, 16.67%) Gram positive cocci and one (1, 8.33%) Gram positive rod. Thus, the bacteria isolates were majorly more of Gram-negative bacteria associated with mosquito abdomen (Tables 2).

Table 1: Total Heterotrophic Bacterial Count (THBC)

Sample	THBC (LogCFU/ml)	SD	SE	p-value
Blood fed 1	6.34	0.23	0.16	
Blood fed 2	6.63	0.24	0.17	0.532
Blood fed 3	6.68	0.10	0.07	
Non-blood fed	6.46	0.17	0.12	

Equivalence testing of the sample means

Table 3 and table 4 shows the Two One-Sided Tests (TOST) for equivalence between the three blood-fed groups and the non-blood fed group to confirm the statistical result of analysis of variance (ANOVA) of no significant difference. For Blood fed 1, TOST gave t-value of -0.594, p-value of 0.613, and the upper and lower bound tests (p-value = 0.619 and p-value = 0.245, respectively); Blood fed 2, the t-value of 0.829 (p-value = 0.494) with upper and lower bound p-values of 0.199 and 0.692, respectively, and Blood fed 3, the t-value was higher at 1.536 (p-value = 0.264) with upper and lower bound p-values of 0.099 and 0.820 show no statistical proof of equivalence in the THBC of blood fed sample from non-blood fed. None of the blood-fed groups were statistically equivalent within the defined limits, meaning the observed differences might be outside the equivalence margin.

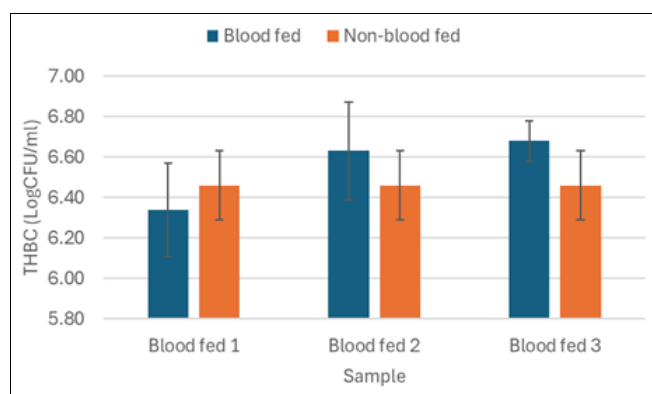
Table 2: Biochemical Identification of Bacteria Isolates from Abdomen of *Anopheles gambiae* s.l Mosquitoes

Isolate Code	Colour	Shape	Size	Elevation	Margin	Opacity	Surface appearance	Surface Texture	Gram Reaction	Catalase	Oxidase	Citrate	Starch Hydrolysis	Urease	Indole	Motility	Methyl red	Voges Proskauer	Glucose	Lactose	Mannose	Maltose	Probable Bacteria
1	Gray-White	I	Large	Flat	Undulate	Opaque	Rough	Dry	+	+	+	+	-	-	-	-	-	+	AG	A	AG	A	<i>Bacillus</i> sp.
2	green	C	Large	Umbonate	Undulate	Opaque	Smooth	Dry	-	+	+	+	-	+	-	+	-	-	N	N	A	N	<i>Pseudomonas aeruginosa</i>
3	Orange	C	Large	Convex	Entire	Translucent	Smooth	Mucoid	+	+	-	+	-	-	-	-	+	+	A	A	A	A	<i>Staphylococcus</i> sp
4	Red	C	Small	Convex	Entire	Opaque	Smooth	Mucoid	-	+	-	+	+	+	-	+	-	+	A	N	N	A	<i>Serratia marcescens</i>
5	Cream	C	raised	Raised	Undulate	Opaque	Rough	Mucoid	-	+	-	+	-	-	-	+	-	+	A	N	A	AG	<i>Enterobacter</i> sp
6	Cream	C	Small	Umbonate	Undulate	Translucent	Smooth	Mucoid	-	+	-	+	-	+	-	-	-	+	A	A	A	AG	<i>Klebsiella</i> sp
7	white	C	Large	Umbonate	Lobate	Opaque	Smooth	Mucoid	-	+	-	+	-	-	-	-	-	-	AG	N	N	A	<i>Acinetobacter</i> sp
8	Orange	C	Large	Convex	Entire	Translucent	Smooth	Mucoid	+	+	-	+	-	-	-	-	+	+	AG	A	A	A	<i>Staphylococcus aureus</i>
9	white	C	Large	Umbonate	Undulate	Opaque	Smooth	Dry	-	+	+	+	-	+	-	+	-	-	N	N	A	N	<i>Pseudomonas</i> sp
10	Cream	C	Small	Raised	Entire	Opaque	Smooth	Mucoid	+	+	-	+	-	-	-	+	+	-	A	A	N	A	<i>Streptococcus</i> sp
11	Cream	C	Small	Raised	Entire	Translucent	Shiny	Mucoid	-	-	-	-	-	-	+	+	+	-	A	AG	A	N	<i>Escherichia coli</i>
12	Cream	C	Small	Convex	Entire	Opaque	Smooth	Mucoid	-	+	-	+	+	+	-	+	-	+	A	N	N	A	<i>Serratia</i> sp

KEYS: GPR = Gram positive rod, GNR = Gram negative rod, GPC = Gram positive cocci, AG = Acid/Gas, A = Acid, N = Negative/Neutral, + = Positive, - = Negative, C=Circular, I=irregular

Table 3: Equivalence Independent Samples T-Test

Sample	Statistic	t-test	p-value
Blood fed 1	T-Test	-0.594	0.613
	Upper bound	-0.347	0.619
	Lower bound	-0.840	0.245
Blood fed 2	T-Test	0.829	0.494
	Upper bound	1.068	0.199
	Lower bound	0.590	0.692
Blood fed 3	T-Test	1.536	0.264
	Upper bound	1.894	0.099
	Lower bound	1.177	0.82



Graph: Mean THBC (LogCFU) values: Blood Fed vs non-Blood Fed

Discussion

This study on isolation, identification and characterization of microorganisms (bacteria) inhabiting the abdomen of *Anopheles gambiae* s.l mosquitoes showed a high bacterial load that ranged from 6.34±0.23 to 6.68±0.10 (LogCFU/ml) (Table 1). This is clear evidence that *Anopheles gambiae* s.l mosquitoes harbour a populous bacterial community in its abdomen. This could be associated with the synergistic

relationship between the bacterial community and *Anopheles gambiae* as endogenous bacteria reduces viral and parasitic infections in mosquito by activating immune responses or inhibiting pathogen development (Dong *et al.*, 2009; Cirimotich *et al.*, 2011). Different genera of mosquitoes have been reported to harbour diverse bacterial community (Chandel *et al.*, 2013; Engel & Moran, 2013; Adly *et al.*, 2022). The occurring diverse bacterial genera of gram-negative and few gram-positive bacteria showed that the abdomen of mosquitoes is an environment inhabitable by several species of microbes, collaborating with studies that reported the presence of enterobacteriaceae and non-enteric bacterial from the mid-gut of mosquitoes (Lindh *et al.*, 2008; Djadid *et al.*, 2011; Prasad *et al.*, 2012; Wu *et al.*, 2019). Gram negative bacteria species have been reported to dominate the abdomen of *Anopheles gambiae* s.l mosquitoes as also observed in this research (Wu *et al.*, 2019; Oliveira *et al.*, 2020). Some of these could hinder the ability of the vector to transmit *Plasmodium* species to man (Oliveira *et al.*, 2020; Tainchum *et al.*, 2020; Rocha *et al.*, 2021).

The nine (9) bacteria genera identified from the abdomen of blood fed and non-blood fed *Anopheles gambiae* s.l mosquitoes were *Pseudomonas* sp., *Staphylococcus* sp., *Streptococcus* sp., *Acinetobacter* sp., *Klebsiella* sp., *Bacillus* sp., *Serratia* sp., *Escherichia coli.*, and *Enterobacter* sp. (Table 2). Their presence could be due to human-mosquito interaction as also reported in some previous studies (Dong *et al.*, 2009; Prasad *et al.*, 2012; Galal *et al.*, 2017) and the breeding sites (Galal *et al.*, 2017; Barko *et al.*, 2018; Hery *et al.*, 2021; Seal & Chatterjee, 2023). These genera of bacteria have also been isolated from the abdomen of *Anopheles* mosquitoes in the studies conducted by Prasad *et al.* (2012), Ngo *et al.* (2016), Oliveira *et al.* (2020) and Rocha *et al.* (2021). There is also research evidence associating *Streptococcus* sp., and *Pseudomonas* sp., *Serratia* sp. and

Enterobacter sp. isolates with blood fed mosquitoes (Habtewold *et al.*, 2016; Oliveira *et al.*, 2020; Tainchum *et al.*, 2020; Suo *et al.*, 2022).

Conclusion

This study has revealed the occurrence of large diversity of bacteria species in the abdomen of *Anopheles gambiae* s.l. mosquitoes. The microbiota characterized included pathogenic gram-positive bacteria and gram-negative bacteria; and gram-negative rods been more abundant. While blood fed *Anopheles gambiae* s.l. had higher microbial load than the non-blood fed *Anopheles gambiae* s.l. mosquitoes. It is also important to elucidate the role of naturally occurring bacteria in *Anopheles* mosquito populations as a potential method of disease control.

References

- Adly E, Hegazy AA, Kamal M, Abu-Hussien SH. Midguts of *Culex pipiens* L. (Diptera: Culicidae) as a potential source of raw milk contamination with pathogens. *Scientific Reports*,2022;12(1):13183.
- Bahia AC, Dong Y, Blumberg BJ, Mlambo G, Tripathi A, BenMarzouk-Hidalgo OJ, *et al.* Exploring *Anopheles* gut bacteria for *Plasmodium* blocking activity. *Environmental Microbiology*,2014;16(9):2980–2994.
- Bando H, Okado K, Guelbeogo WM, Badolo A, Aonuma H, Nelson B, *et al.* Intra-specific diversity of *Serratia marcescens* in *Anopheles* mosquito midgut defines *Plasmodium* transmission capacity. *Scientific Reports*,2013;3:1641.
- Barko PC, McMichael MA, Swanson KS, Williams DA. The gastrointestinal microbiome: A review. *Journal of Veterinary Internal Medicine*,2018;32(1):9–25.
- Berhanu A, Abera A, Nega D, Mekasha S, Fentaw S, Assefa A, *et al.* Isolation and identification of microflora from the midgut and salivary glands of *Anopheles* species in malaria endemic areas of Ethiopia. *BMC Microbiology*,2019;19(1):85.
- Chandel K, Mendki MJ, Parikh RY, Kulkarni G, Tikar SN, Sukumaran D, *et al.* Midgut microbial community of *Culex quinquefasciatus* mosquito populations from India. *PLoS ONE*,2013;8(11):e80453.
- Cirimotich CM, Dong Y, Clayton AM, Sandiford SL, Souza-Neto JA, Mulenga M, *et al.* Natural microbe-mediated refractoriness to *Plasmodium* infection in *Anopheles gambiae*. *Science*,2011;332:855–858.
- Cirimotich CM, Ramirez JL, Dimopoulos G. Native microbiota shape insect vector competence for human pathogens. *Cell Host and Microbe*,2011;10(4):307–310
- Cox FE. History of the discovery of the malar 23 parasites and their vectors. *Parasites and Vectors*,2010;3(1):5.
- Djadid DN, Jazayeri H, Raz A, Favia G, Ricci I, Zakeri S. Identification of the midgut microbiota of *Anopheles stephensi* and *Anopheles maculipennis* for their application as a paratransgenic tool against malaria. *PLoS ONE*,2011;6(12):1–7.
- Dong Y, Manfredini F, Dimopoulos G. Implication of the mosquito midgut microbiota in the defense against malaria parasites. *PLoS Pathogens*,2009;5(5):e1000423.
- Engel P, Moran NA. The gut microbiota of insects: diversity in structure and function. *FEMS Microbiology Reviews*,2013;37(5):699–735.
- Galal FH, Abuelnasr A, Abdallah I, Zaki O, Seufi AM. *Culex* (*Culex*) *pipiens* mosquitoes carry and harbor pathogenic fungi during their developmental stages. *Erciyes Medical Journal*,2017;39(1):1–6.
- Gillies M, Coetzee M. A supplement to the *Anophelinae* of Africa south of the Sahara. Publications of the South African Institute for Medical Research, 1987, 55–143.
- Gillies M, De Meillon B. The *Anophelinae* of Africa south of the Sahara (Ethiopian zoogeographical region). Publications of the South African Institute for Medical Research,1968;54:314.
- Habtewold T, Duchateau L, Christophides GK. Flow cytometry analysis of the microbiota associated with the midguts of vector mosquitoes. *Parasites and Vectors*,2016;9(1):1–10.
- Hery L, Guidez A, Durand AA, Delannay C, Normandeau-Guimond J, Reynaud Y, *et al.* Natural variation in physicochemical profiles and bacterial communities associated with *Aedes aegypti* breeding sites and larvae. *Microbial Ecology*,2021;81(1):93–109.
- Lindh JM, Kannaste A, Knols BG, Faye I, Borg-Karlson AK. Oviposition response of *Anopheles gambiae* s.s. and identification of volatiles from bacteria-containing solutions. *Journal of Medical Entomology*,2008;45(6):1039–1049.
- Manguin S, Ngo CT, Tainchum K, Juntarajumnong W, Chareonviriyaphap T, Michon AL. *et al.* Bacterial biodiversity in midguts of *Anopheles* mosquitoes. *Anopheles Mosquitoes: New Insights into Malaria Vectors*, 2013, 549–576.
- Ngo CT, Romano-Bertrand S, Manguin S, Jumas-Bilak E. Diversity of the bacterial microbiota of *Anopheles* mosquitoes from Binh Phuoc Province, Vietnam. *Frontiers in Microbiology*,2016;7:2095.
- Oliveira TMP, Sanabani SS, Sallum MAM. Bacterial diversity associated with *Nyssorhynchus darlingi*. *BMC Microbiology*,2020;20(1):180.
- Osei-Poku J, Mbogo CM, Palmer WJ, Jiggins FM. Deep sequencing reveals extensive variation in the gut microbiota of wild mosquitoes from Kenya. *Molecular Ecology*,2012;21:185.
- Poly N, Mamtaz S, Khan M, Hoque M, Azad A, Hasan M. *et al.* Isolation and biochemical characterization of cellulolytic bacteria from rumen fluid of cattle. *Journal of Advanced Biotechnology and Experimental Therapeutics*,2022;5(2):433–444.
- Prasad A, Kumar DS, Megha SE, Priyansh M. Soil bacteria and their possible role in mosquito control. *World Journal of Environmental Biosciences*,2012;2(1):40–48.
- Rocha EM, Marinotti O, Serrao DM, Correa LV, Katak RM, de Oliveira JC, *et al.* Culturable bacteria associated with *Anopheles darlingi* and their paratransgenesis potential. *Malaria Journal*,2021;20(1):40.
- Seal M, Chatterjee S. Combined effect of physico-chemical and microbial quality of breeding habitat water on oviposition of *Anopheles subpictus*. *PLoS ONE*,2023;18(3):e0282825.

27. Service MW. Mosquitoes (Culicidae): medical importance. *Medical Insects and Arachnids*,1993;5:196–208.
28. Sharma P, Sharma S, Maurya RK, Das DT, Thomas T, Lata S. *et al.* Salivary glands harbor more diverse microbial communities than gut in *Anopheles culicifacies*. *Parasites and Vectors*,2014;7:235.
29. Straif SC, Mbogo CN, Toure AM, Walker ED, Kaufman M. Midgut bacteria in *Anopheles gambiae* and *Anopheles funestus*. *Journal of Medical Entomology*,1998;35:222–226.
30. Suo P, Wang K, Yu H, Fu X, An L, Bhowmick B, *et al.* Seasonal variation of midgut bacterial diversity in *Culex quinquefasciatus*. *Biology*, 2022, 11(8).
31. Tainchum K, Dupont C, Chareonviriyaphap T, Jumas-Bilak E, Bangs MJ, Manguin S. Bacterial microbiome in wild-caught *Anopheles* mosquitoes in western Thailand. *Frontiers in Microbiology*,2020;11:965.
32. Tambuwal AD, Muhammad IB, Alhaji S, Muhammad S, Ogbiko C. Morphological and biochemical characterization of fungi and bacteria from indigenous sources. *GSC Biological and Pharmaceutical Sciences*,2018;5(3):86–94.
33. Ulfat M, Abad Z, Ali NM, Sarwar S, Jabeen K, Abrar A. *et al.* Screening and biochemical characterization of bacteria from soil and water samples. *Brazilian Journal of Biology*,2022;84:e254016.
34. Wu P, Sun P, Nie K, Zhu Y, Shi M, Xiao C, *et al.* A gut commensal bacterium promotes mosquito permissiveness to arboviruses. *Cell Host and Microbe*,2019;25(1):101–112.